# STIMULATION OF BACTERICIDAL ACTIVITY OF HUMAN NEUTROPHILS BY INTERLEUKIN-2-ACTIVATED LYMPHOID CELLS: EFFECT OF PROINFLAMMATORY CYTOKINES

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**KEY WORDS:** bactericidal activity of neutrophils; lymphocytes; macrophages; interleukin-2; proinflammatory cytokines.

Bactericidal activity of neutrophils (BAN) is under the control of lymphocytes and monocytes and also of cytokines secreted by them [3, 6]. The writers previously observed a decrease in the ability of mononuclear cells to potentiate BAN when stimulated by interleukin-2 (IL-2) [14]. The aim of the present investigation was to study the possibility of correcting the neutrophil-stimulating function of lymphoid cells (unfractionated mononuclear cells or lymphocytes, freed from monocytes), activated by IL-2, including with the aid of interleukin-1$\beta$ (IL-1$\beta$), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and interferon-$\gamma$ (IFN-$\gamma$).

## EXPERIMENTAL METHOD

Peripheral blood neutrophils were isolated from healthy blood donors by a modified method in [9]. The method consisted of three stages: a) sedimentation of the erythrocytes with dextran; b) removal of lymphocytes, monocytes, and platelets on a Ficoll-Verografin density gradient (d = 1.077 g/cm$^3$); c) isolation of neutrophils on a double Percol gradient ("Pharmacia") with densities of 1.0779 and 1.0945 g/cm$^3$. The highly purified neutrophils thus obtained (98.6% purity) contained about 0.4% of mononuclear cells but no contaminating erythrocytes or platelets. Mononuclear cells were obtained from healthy human blood on a Ficoll-Verografin density gradient [5]. To remove monocytes, mononuclear cells ($5 \cdot 10^6$-$7 \cdot 10^6$/ml) in medium 199 containing 10% heat-inactivated group IV blood serum (AB-serum) were treated with iron carbonyl (IC, "Sigma," 20 mg/ml). After incubation for 1 h at 37°C cells undertaking phagocytosis of IC were removed by means of a magnet. Under these circumstances the percentage of cells giving a positive reaction for nonspecific $\alpha$-naphthyl acetate esterase (a biochemical marker of monocytes) or CD 14 (a phenotypic marker of monocytes) fell from 10-15% to 0-1%. The lymphoid cells were activated with IL-2 for 3 days in an atmosphere of air with 7.5% $CO_2$ in medium RPMI 1640 ("Gibco"), containing 5% of AB-serum and antibiotics. Recombinant IL-2 (rIL-2, "Getus") was added up to a concentration of 1000 IU/ml. The bactericidal activity of the neutrophils was estimated by a micromethod [13] relative to *Staphylococcus aureus* strain SG 511 cells, obtained from the State Institute for Pure Cultures, Ministry of Health of the Russian Federation. The bacteria were given preliminary treatment with ultrasound and were opsonized in medium RPMI-1640 containing 10% AB serum. The neutrophils were incubated for 15-20 min in medium RPMI-1640 with 10% AB-serum and with staphylococci in the ratio of 1:10. The neutrophils were then washed twice to remove unbound bacteria and incubated for 4 h in the presence of allogeneic lymphoid cells in the ratio of 2:1, with or without IL-1$\beta$, TNF-$\alpha$, and IFN-$\gamma$. Recombinant IL-1$\beta$ (rIL-1$\beta$) used in the experiments was obtained from the All-Union Research Institute of High-Purity Bacterial Products, Ministry of the Medical and Biological Industry of the Russian Federation, St. Petersburg, with activity of

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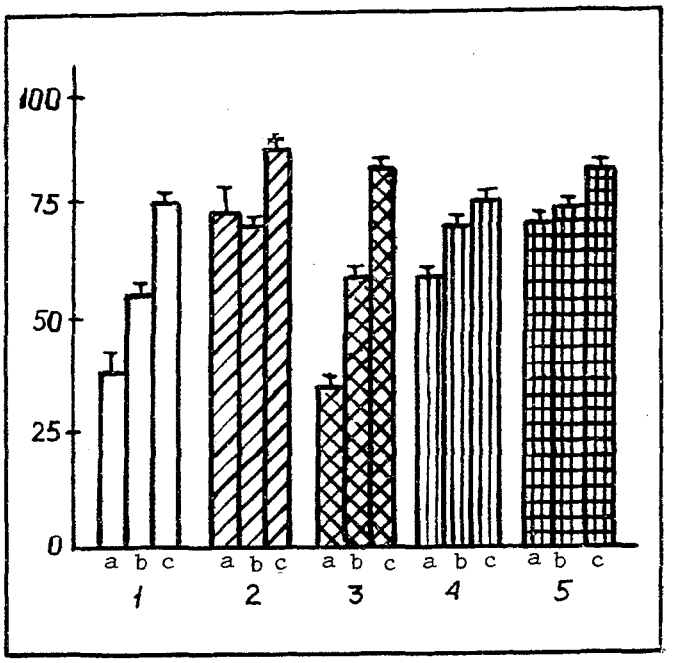


Fig. 1. Effect of lymphoid cells on bactericidal activity of human neutrophils. Ordinate, index of bactericidal activity (in %); abscissa, neutrophils (1); neutrophils + mononuclear cells (2); neutrophils + mononuclear cells activated by interleukin-2 (3); neutrophils + lymphocytes (4); neutrophils + lymphocytes activated by interleukin-2 (5). Results of investigation of three healthy donors with different initial levels of bactericidal activity of their neutrophils are shown (a, b, c), typical of groups from 7, 11, and 11 persons respectively Significance of differences: $p_{1-2} < 0.05$, $p_{1-4} < 0.05$ (a, b); $p_{2-3} < 0.05$ (a, b), $p_{4-5} < 0.05$ (a, b).

$5 \cdot 10^4$ U/ml, recombinant TNF-$\alpha$ (rTNF-$\alpha$), and recombinant IFN-$\gamma$ (rIFN-$\gamma$), from the Ferment Research-Production Combine (Vilnius), with activity of $3 \cdot 10^4$ and $1 \cdot 10^6$ U/ml respectively, were used in the experiments. At the end of incubation the supernatants were removed and the cells were lysed with distilled water. The cell lysate with surviving bacteria was transferred into the wells of another microplanchet, containing nutrient broth with 0.5% glucose. After mixing, seedings were made on Petri dishes containing nutrient agar. The dishes were incubated for 24 h at 37°C, after which the bacterial colonies were counted visually. BAN was characterized by the bactericidal index (BI), calculated by the equation in [8]:

$$BI = (1 - K_4/K_0) \times 100\%,$$

where $K_4$ is the number of living bacteria (colony-forming units) after incubation for 4 h with neutrophils, and $K_0$ the number of living bacteria after mixing with neutrophils.

The numerical results were subjected to statistical analysis by the usual methods. The significance of differences between the values compared was determined by Student's t test.

TABLE 1. Effect of Proinflammatory Cytokines on Bactericidal Activity of Neutrophils in Presence of Human Lymphocytes

| No. of experiment | Bactericidal index of neutrophils (%), incubated with | | | | |
|---|---|---|---|---|---|
| | control | lymphocytes | lymphocytes activated by IL-2 and proinflammatory cytokines in concentration of | | |
| | | | 0 | 100 U/ml | 1000 U/ml |
| 1 | 48,0±1,0 | 60,2±3,5 | 67,0±1,6 | 75,2±2.9 | 75,4±1,6* |
| 2 | 80,5±1,4 | 93,4±0,5 | 95,7±0,3 | 92,6±1,5 | 93,7±1,3 |
| 3 | 43,3±1,7 | 68,2±2,5 | 68,5±2,7 | 74,8±1,6 | 73,8±1,2 |
| 4 | 68,0±2,2 | 72,3±1,2 | 71,7±2,2 | 77,1±4,2 | 60,8±1,7 |
| 5 | 26,7±4,4 | 72,8±1,6 | 72,0±1,3 | 86,1±1,3* | 75,5±4,5 |
| 6 | 61,3±0,6 | 71,3±0,6 | 70,2±1,0 | 70,6±2,3 | 72,8±2,3 |

**Legend.** Recombinant IL-1$\beta$ was used in experiments 1 and 2, recombinant TNF-$\alpha$ in experiments 3 and 4, and recombinant IFN-$\gamma$ in experiments 5 and 6.

*$p < 0.05$ Compared with level of BAN in presence of lymphocytes.

## EXPERIMENTAL RESULTS

We first tested the bactericidal activity of neutrophils from healthy blood donors. As Fig. 1 shows, it varied within wide limits, but was significantly increased in the presence of mononuclear cells or purified lymphocytes in 23 of the 29 experiments carried out. Additional experiments and investigations by other workers showed that such an increase could not be due to the direct bactericidal action of the added mononuclear cells or the purified lymphocytes, whose bactericidal activity is only one-tenth as strong as that of neutrophils [1]. Purified lymphocytes caused potentiation of BAN by a lesser degree than mononuclear cells. We attribute this to the presence of 10-15% of monocytes, which have a direct bactericidal action and can secrete IL-8 and other neutrophil-stimulating monokines [11], in the mononuclear cells. The fact will be noted (Fig. 1, Table 1) that the degree of potentiation of BAN under the influence of lymphoid cells depends directly on the initial level of bactericidal activity of the neutrophils. The lower the initial level of BAN, the greater the degree of its increase in the presence of lymphoid cells. The higher the initial level of BAN, moreover, the smaller its increase in the presence of lymphoid cells. In six experiments we studied the effect of activation by IL-2 on the neutrophil-stimulating ability of the lymphoid cells. In three experiments mononuclear cells activated by IL-2 caused suppression of BAN compared with unactivated cells. In other experiments with a high initial level of BAN the neutrophil-stimulating activity of activated and unactivated mononuclear cells did not differ significantly. Unlike mononuclear cells, IL-2-activated lymphocytes purified from monocytes significantly potentiated BAN by comparison with unactivated cells (Fig. 1). This is evidence that monocytes, stimulated by IL-2, can induce suppression of the neutrophil-stimulating function of lyphocytes.

In preliminary experiments we showed that recombinant IL-1$\beta$, TNF-$\alpha$, and INF-$\gamma$ enhance the bactericidal activity of blood neutrophils of healthy donors if its initial level is low, but do not affect BAN if its initial level is normal (65-90%). We estimated the action of the above-mentioned proinflammatory cytokines on BAN in the presence of lymphocytes activated or not activated by IL-2. In this series of experiments we used lymphocytes freed from monocytes, for an activating effect of IL-1$\beta$, TNF-$\alpha$, and IFN-$\gamma$ on macrophagal function is known [3, 11]. As Table 1 shows, in the presence of lymphocytes (not activated or activated by IL-2) the action of proinflammatory cytokines depended on the initial level of BAN was over 50%, addition of IL-1$\beta$, TNF-$\alpha$, and IFN-$\gamma$ together with lymphocytes did not change the parameters of BAN significantly compared with the addition of lymphocytes alone. If the initial level of BAN was below 50%, it was increased not only in the presence of lymphocytes, but also additionally (significantly in most experiments) and in the presence of proinflammatory cytokines.

The study of cellular and cytokine-dependent mechanisms of regulation of BAN thus revealed, in the first place, an important role of lymphocytes in neutrophil stimulation. Activated (by interleukin-2) monocytes play the role of suppressors of the neutrophil-stimulating function of the lymphocytes, as has been observed on other models also [2, 7].

Another important problem is that of cytokine-dependent regulation of BAN in the presence of IL-2-activated lymphocytes. We found that cytokines have a stimulating action mainly on the depressed function (bactericidal activity) of neutrophils We also know that activated lymphocytes themselves secrete cytokine, including IL-1$\beta$, TNF-$\alpha$, and IFN-$\gamma$ [4, 12]. In that case potentiation of the functions of neutrophils in our experiments may be due to the co-stimulating action of exogenous and endogenous cytokines. The ability of cytokines to enhance the adhesiveness of cells and to increase the resistance of cell membranes to cytokines of different origin has recently been demonstrated [10], and this is a matter of importance for phagocytosis of bacteria by short-living neutrophils.

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